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Cultured mesangial cells from autoimmune MRL-*lpr* mice have decreased secreted and surface M-CSF

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Cultured mesangial cells from autoimmune MRL-*lpr* mice have decreased secreted and surface M-CSF. M-CSF has been implicated in the pathogenesis of lupus nephritis in MRL-*lpr* mice. We recently reported persistently high levels of serum M-CSF in MRL-*lpr* mice as early as one week of age, not present in normal mice including C3H mice. In addition, M-CSF transcripts in MRL-*lpr* renal cortex increased with an increase in the severity of nephritis. Because glomerular mesangial cells (MC) secrete M-CSF, we investigated whether cultured MRL-*lpr* MC secrete more M-CSF than C3H MC. Paradoxically, unstimulated MRL-*lpr* MC secreted substantially less M-CSF than C3H MC [26 ± 11 vs. 109 ± 7 colony forming units (CFU)]. We then explored whether MC could express membrane bound M-CSF. We detected a 31 kDa form of membrane M-CSF on both MRL-*lpr* and C3H MC. Fewer MRL-*lpr* MC than C3H MC (24 ± 5% vs. 78 ± 5%) expressed membrane M-CSF. Furthermore, the increase in the mean channel log fluorescence intensity on MRL-*lpr* MC was considerably less than in C3H MC, indicating a lower density of M-CSF on MRL-*lpr* MC. Because our prior studies established that MRL-*lpr* kidneys have enhanced expression of TNFα, we stimulated cultured MC with TNFα. TNFα increased M-CSF secretion by stimulated MRL-*lpr* by twofold over unstimulated MRL-*lpr* MC, but did not increase M-CSF in C3H MC. In addition, M-CSF secretion was modestly greater in stimulated MRL-*lpr* MC compared to stimulated C3H MC. In conclusion, this is the first report of membrane M-CSF detectable on cultured MC. These studies note that despite higher circulating M-CSF and renal M-CSF transcripts in MRL-*lpr* mice, cultured MRL-*lpr* MC have lower basal secreted and membrane bound M-CSF than cultured C3H MC. TNFα increases secretion of M-CSF by MRL-*lpr* MC, but does not enhance membrance M-CSF. Since these studies are restricted to a comparison of MC from autoimmune and normal mice in vitro, we are engaged in further studies to evaluate in vivo production of M-CSF by MC and the importance of this growth factor in renal injury.

M-CSF (macrophage-colony stimulating factor) is a hematopoietic cytokine that is a chemoattractant for macrophages [1] as well as a growth and differentiation factor [2] for macrophages. MRL-*lpr* mice are a model of systemic lupus erythematosus (SLE) and develop an aggressive lupus nephritis. Progressive kidney disease is associated with mesangial cell proliferation and accumulation of macrophages in the renal glomerulus and increased levels of mRNA for the pro-inflammatory cytokines TNFα and IL-1β in the renal cortex [3, 4]. Recently, we reported that MRL-*lpr* mice have an increased level of circulating M-CSF [colony stimulating factor-1 (CSF-1)], which may augment macrophage mediated renal injury [5]. M-CSF was detectable in MRL-*lpr* mice as early as one week of age, decreased between two to four months of age, and then increased to the highest levels beginning at four months of age. Circulating M-CSF was not detected in sera from congenic MRL-++, normal C3H/FeJ (C3H), or two other mouse strains with the *lpr* gene. Consistent with serum data, M-CSF transcripts were elevated in the kidneys of MRL-*lpr* mice, but not normal C3H/FeJ or congenic MRL-++ mice with normal renal function [5]. These results suggest that local production of M-CSF as well as systemically increased levels may be important for the pathogenesis of lupus nephritis.

The factors which regulate M-CSF production within lupus kidneys are not clear, as mesangial cells from normal and autoimmune mice express this cytokine [6, 7]. Using a semi-solid agar colony stimulating assay, we examined whether M-CSF production capacity differed between mouse strains. Since several cytokines, including IL-1 and TNF, are membrane bound [8–10], we also investigated whether mesangial cells (MC) from normal and autoimmune strains have a surface form of M-CSF. Paradoxically, although MRL-*lpr* have circulating levels of M-CSF not detected in normal mice, unstimulated MRL-*lpr* MC secreted less M-CSF than normal C3H MC. However, MRL-*lpr* MC were readily induced by TNF to increase M-CSF production, while C3H MC were unresponsive to this stimulus. Antibody neutralization assays indicated that secreted colony stimulating factor was exclusively M-CSF and not GM-CSF. We now report the presence of membrane M-CSF on MC. Again, contrary to our expectations, the percentage of unstimulated MRL-*lpr* MC expressing M-CSF was much less than the percentage of unstimulated C3H MC expressing M-CSF as analyzed by flow cytometry. However, surface expression of M-CSF was not increased with TNFα stimulation in either MRL-*lpr* or C3H MC. Western blot analysis of cell lysates revealed the existence of a 31 kDa form of M-CSF on MC. We hypothesize that membrane and secreted M-CSF act to recruit and stimulate macrophages to proliferate in the glomerulus. In turn, the accumulation of macrophages in

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the glomerulus can generate molecules known to promote renal injury.

Methods

Mice

Normal C3H/FeJ (H-2^k) (C3H) and C3H/HeN (H-2^k), autoimmune MRL/MpJ-*lpr/lpr* (MRL-*lpr*) (H-2^k), and MRL/MpJ-++ (MRL-++) (H-2^k) female mice were obtained from The Jackson Laboratory (Bar Harbor, Maine, USA). All animals were maintained in our animal facility on standard laboratory chow.

Reagents

Tissue culture media and reagents were from Grand Island Biological Company (New York, USA). Chemicals were purchased from Sigma (St. Louis, Missouri, USA) unless otherwise indicated. Polyclonal rabbit anti-mouse L cell-derived M-CSF (anti-M-CSF antibody) was provided by Dr. R. Shadduck, Montefiore Hospital, Pittsburgh, Pennsylvania, USA. ^{125}I-iodinated goat anti-rabbit polyclonal antibody was a gift from Dr. S. Greenberg, Harvard Medical School (Boston, Massachusetts, USA). Normal rabbit serum was purchased from Calbiochem (Behring Diagnostics, La Jolla, California, USA). Murine rTNFα (0.98 mg/ml, 1.2×10^7 μ/mg was provided by Genentech (South San Francisco, California, USA). Mouse L929 cells were obtained from American Type Culture Center (Rockville, Maryland, USA). Mouse CH33 cells (B cell tumor cells derived from B10.A mice) were a gift from Dr. D. Perkins, Harvard Medical School (Boston, Massachusetts, USA).

Mesangial cell isolation and growth

Untransformed MC lines were established and characterized as previously described [11, 12] using collagenase dispersion of sieved glomeruli from C3H/FeJ, MRL-*lpr* or MRL-++ mice that were between two to four months of age. Cell monolayers were treated with trypsin (0.025%) EDTA (0.5 mM) and passaged as needed in high (15 to 20%) fetal calf serum (FCS) containing media.

Colony stimulating assays

M-CSF was assessed as previously described [5]. Bone marrow cells were obtained from the tibias of 20 g male C3H/HeN or C3H/FeJ mice and cultured in duplicate in soft agar [13]. Briefly, 1×10^5 bone marrow cells were added to 30 μl test serum or 200 μl supernatant (SN) and plated in 1 ml 0.3% Noble agar (Difco, Detroit, Michigan, USA) in McCoy's 5A medium (Gibco) supplemented with 15% fetal calf serum (FCS; Hyclone, Logan, Utah, USA), 50 μM 2-mercaptoethanol (Sigma), 10 U/ml penicillin and 10 μg/ml streptomycin (Gibco) in 35 mm tissue culture plates (Costar, Cambridge, Massachusetts, USA). Cultures were incubated at 37°C in a humidified 7% CO_2 atmosphere and the colonies counted on day 10 to 12 with a dissecting microscope. Colonies at this time point are macrophage colonies as granulocytic colonies do not persist this late in culture [14]. Results of colony-stimulating activity (CSA) are reported as colony forming units (CFU)/10^5 bone marrow cells. This assay has a limit of detection of 50 units of M-CSF based on determination of CFU using murine r-M-CSF. For inhibition studies, 30 μl of test sera or 200 μl of culture supernatant was preincubated with 0.1 to 0.2 ml of various dilutions of either polyclonal rabbit anti-mouse L cell-derived M-CSF or normal rabbit serum for 30 minutes at room temperature before assay. The specificity of M-CSF in sera and supernatant was tested by preincubation with M-CSF-containing L cell supernatant, recombinant murine granulocyte macrophage CSF (rGM-CSF) (Genzyme, Cambridge, Massachusetts, USA) and murine recombinant interleukin-3 (rIL-3) (Biogen, Cambridge, Massachusetts, USA), as previously described [5].

Flow cytometric analysis

Monolayers of MC were gently trypsinized to produce a single-cell suspension and labeled using the specific polyclonal rabbit anti-M-CSF at optimum concentrations. Controls included cells without primary antibody or stained with irrelevant normal rabbit serum at an equivalent dilution to the anti-M-CSF antibody. Fluorescein (FITC)-conjugated affinity-purified goat anti-rabbit IgG F(ab')$_2$ was used as a secondary reagent. Cells were fixed in 2% paraformaldehyde and analyzed on an Epics flow cytometer (Coulter Electronics, Hialeah, Florida, USA).

Immunoprecipitation and Western blot for surface M-CSF

Confluent cell monolayers were trypsinized, washed three times in HBSS and then lysed in 1 ml of extract buffer containing 1% Nonidet P-40 (NP-40), 50 mM KCl, 10 mM NaCl, 1 mM EDTA, 50 mM KH_2PO_4 buffer (pH 7.5), 2 mM phenylmethylsulfonyl fluoride (PMSF), and 2 μg/ml of antipain, leupeptin and pepstatin A. Debris was removed by centrifugation at 15,000 rpm for 20 minutes and then an aliquot of the lysate was precleared with normal rabbit serum and Protein-A Sepharose for 16 hours. Half of the precleared lysate was immunoprecipitated by incubation with a 1/100 dilution of anti-M-CSF antisera for 2 hours, followed by precipitation with Protein-A Sepharose beads for one hour. The immunoprecipitate was washed three times with lysis buffer and then twice with PBS pH 7.2. Buffer containing SDS without reducing agents was added to the samples, and after boiling for two minutes, 10 μl of either cell lysate extracts, pre-cleared lysates, immunoprecipitated lysates or culture supernatant was electrophoresed through a 10 to 20% SDS polyacrylamide gradient gel or a 12% stacking gel, depending on the particular experiment [15]. The proteins were transferred to nitrocellulose using standard techniques under reducing conditions. Blots were incubated with rabbit anti-M-CSF antisera (1:500), followed by goat anti-rabbit alkaline phosphatase (1:3000) and developed with 0.3 mg/ml *p*-nitro blue tetrazolium chloride plus 0.15 mg/ml 5-bromo-4-chloro-3-indolyl phosphate-toluidine salt. Alternatively, blots were incubated with ^{125}I-iodinated goat anti-rabbit polyclonal serum (1:3000) for one hour following incubation with rabbit anti-M-CSF. Blots were extensively washed and autoradiography with an intensifying screen was carried out at −70°C.

Statistical analysis

Statistical analysis was performed with the Statview SE+ package (Abacus Concepts, Berkeley, California, USA). Treatment groups were compared by one way analysis of variance. All results are expressed as mean ± SD.

Table 1. Unstimulated MRL-*lpr* mesangial cells secrete less M-CSF

Hours	CFU	
	C3H	MRL-*lpr*
48	36 ± 5 (13.8)[a]	14 ± 1 (6.7)
72	109 ± 7 (3.1)	26 ± 11 (0.2)
96	108 ± 6 (8.6)	16 ± 6 (0.9)

Mesangial cells were plated at 1×10^5 cells/well and supernatants assayed for M-CSF at three time points from three separate experiments. M-CSF was measured as colony forming units (CFU) per 10^5 bone marrow cells in semisolid agar. Results are expressed as mean ± SD. The standard in this assay was sera from a C3H/HeN mouse injected with LPS six hours before sera collection (121 CFU/10^5 bone marrow cells) and mouse rGM-CSF (5 ng/ml) (65 CFU/10^5 bone marrow cells).

[a] To normalize these values for differential cell counts, results are expressed as CFU/MC $\times 10^{-5}$ viable cells. Viability was assessed by Trypan blue exclusion.

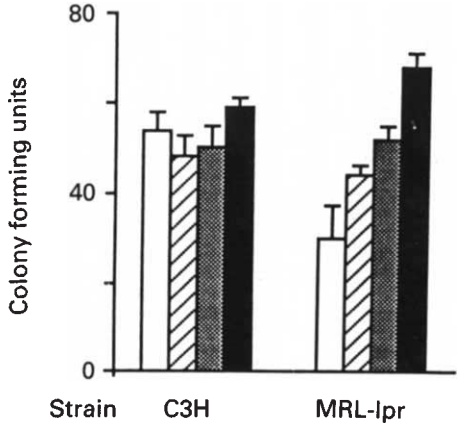

Fig. 1. *TNFα increases secreted M-CSF in MRL-*lpr *MC.* Mesangial cells from C3H or MRL-*lpr* mice were stimulated with various concentrations of TNFα for 24 hours: (□) media; (▨) TNF 0.01 ng; (▩) TNF 1.0 ng; (■) 10 ng. Supernatants were harvested, stored at −20°C and then assayed in duplicate for the presence of M-CSF. This value represents means ± SD of duplicate experiments. This data represent 1 of 2 similar experiments.

Results

*Unstimulated MRL-*lpr *MC mice secrete less M-CSF than C3H MC, but TNFα increases secretion by MRL-*lpr *MC and not by C3H MC*

For these experiments, confluent monolayers of MC were trypsinized and 1×10^5 cells in 2 ml of media were added to each well of a six well tissue culture plate (Falcon, Cambridge, Massachusetts, USA). At 48, 72 and 96 hours after plating, supernatants were obtained from the confluent monolayers and the cells were trypsinized and counted. Viable cells were determined by trypan blue exclusion. Secreted M-CSF activity was determined from the supernatants in a separate CSA assay, and surface expression was analyzed using flow cytometry.

At all time points, secreted M-CSF was lower in MRL-*lpr* MC than C3H MC (Table 1). This difference remained whether the M-CSF was assessed as the total M-CSF or whether the M-CSF was normalized for the number of viable cells at the time the supernatant was obtained.

We also stimulated the cultured MC for 24 hours with TNFα at various concentrations and compared secreted M-CSF in C3H and MRL-*lpr* stimulated and unstimulated MC. TNFα increased M-CSF secretion in the MRL-*lpr* MC, but not the C3H MC (Fig. 1). In the MRL-*lpr*, TNFα 10 ng/ml stimulation increased M-CSF secretion by twofold above unstimulated cells (68 ± 3 vs. 30 ± 7), but did not increase M-CSF secretion in C3H MC (59 ± 2 vs. 54 ± 4) (Fig. 1). The maximal amount of M-CSF secreted was also modestly greater in stimulated MRL-*lpr* MC compared to C3H MC (68 ± 3 CFU vs. 59 ± 2 CFU). This result was confirmed in a second experiment (41 ± 4 CFU for C3H MC vs. 31 ± 0 CFU for MRL-*lpr* MC, $P \leq 0.05$).

*Autoimmune MRL-*lpr *express less surface M-CSF than normal C3H mice*

Surface expression is not regulated by TNFα. Indirect flow cytometric analysis of trypsinized cells was used to assess surface expression of M-CSF. Surface expression was less in the MRL-*lpr* MC than the C3H MC and constant between 48 to 96 hours. The percentage of MRL-*lpr* MC positive for surface M-CSF (24 ± 5%) was one-third that of the C3H MC (78 ± 5%); (Table 2). To determine whether the differences among the cell lines were unique to our specific cell lines, we cultured out fresh MC lines from a second C3H/FeJ mouse and a congenic MRL-++ mouse, which were 2 to 4 months of age. It should be noted that MRL-++ are normal and do not develop renal injury during the first year of life [16]. The secondary cell lines were passaged at least five times to eliminate other contaminating cell types and to increase the homogeneity of the cell lines. In this experiment, 32% of the original MRL-*lpr* MC and 14% of the secondary MRL-*lpr* cell line were positive for surface M-CSF (Fig. 2). These amounts were less than the original C3H, secondary C3H, and the MRL-++ MC lines, in which 67, 70, and 59% of cells were positive for surface M-CSF. In addition, the mean log fluorescence intensity was much less in the MRL-*lpr* MC indicating that not only is the percentage of cells positive for M-CSF less, but the density of surface M-CSF is much less, as well.

Table 2. Cell surface M-CSF is expressed by fewer MRL-*lpr* MC

Strain	Positive %
C3H	78 ± 5
MRL-*lpr*	24 ± 5
L929	98 ± 1
CH33	2 ± 0

Confluent monolayers were trypsinized, plated at 1×10^5 cells/well, grown for 48 to 96 hours then trypsinized again, stained for surface M-CSF and assayed by flow cytometry. Results are mean ± SD of three experiments. $P < 0.01$ C3H versus all groups and MRL-*lpr* versus all groups.

We next determined the role of TNFα in the regulation of surface expression of M-CSF. By flow cytometric analysis, there was not a concomitant increase in membrane M-CSF with TNFα stimulation in either the MRL-*lpr* or the C3H MC (Table 3). These observations are in contrast with the ability of TNFα to induce M-CSF secretion and suggest that TNFα may alter release of intracellular stores or induce more rapid turnover of surface M-CSF in the stimulated MRL-*lpr* MC as compared to normal MC.

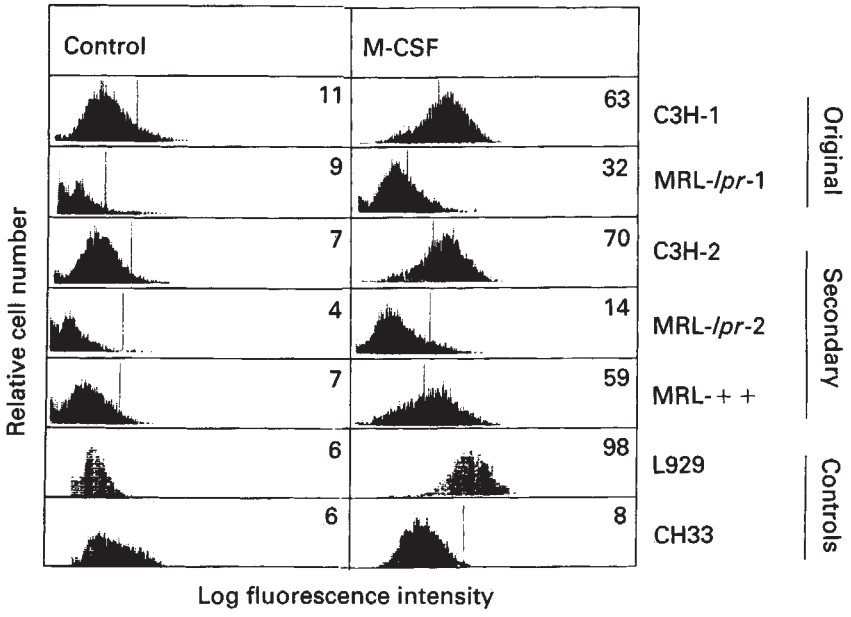

Fig. 2. *Mesangial cells from MRL-*lpr *mice express less surface M-CSF.* Mesangial cells were cultured from C3H and MRL-*lpr* mice, trypsinized and stained for the presence of M-CSF. A secondary cell line was established from different mice and stained similarly. L929 cells were used as a positive control and CH33 as a negative cell line. The column under control is background fluorescence and the % of cells positive is shown in the upper right hand corner. One of at least two similar experiments is shown. Background fluorescence intensity is marked by the cursor and differs between different cell types, but was not altered by the antibody within each individual cell type.

Table 3. TNF does not induce surface M-CSF expression on mesangial cells

	Experiment 1 Positive %		Experiment 2	
	Strain		Strain	
Treatment	C3H	MRL-*lpr*	C3H	MRL-*lpr*
Media	83	28	67	32
TNF *ng/ml*				
0.01	75	24	61	23
1.00	94	18	57	30
10.00	87	19	56	30

Confluent monolayers were trypsinized, plated at 1×10^5 cells/well, grown for 24 hours then trypsinized again, stained for surface M-CSF and assayed by flow cytometry.

Table 4. Neutralization of mesangial cell supernate CSF activity with anti-M-CSF

	CSF Activity (^{3}H-CPM)[a]				
	Media	N.R. serum[b]	Anti-M-CSF[c]	N.G. serum[d]	Anti-GM-CSF[e]
C3H[f]	27,116	21,863	193	30,173	25,427
MRL-*lpr*[g]	7,176	6,957	125	5,791	5,516
L-Cell[h]	7,226	7,519	103	6,890	7,368
mrGM-CSF[i]	44,631	54,762	49,850	47,433	183

[a] CSF Activity measured by the proliferation (^{3}H-CPM incorporation) of normal murine bone marrow cells after three days culture in the presence of 50 μl of test sample, 50 μl of anti-serum or media plus 100,000 normal bone marrow cells

[b] N.R. serum, normal rabbit serum (final dilution of 1:320)

[c] Anti-M-CSF, rabbit anti-murine M-CSF serum (final dilution of 1:320)

[d] N.G. serum, normal goat serum (final dilution of 1:320)

[e] Anti-GM-CSF, goat anti-murine GM-CSF serum (final dilution of 1:320)

[f] C3H cell supernatant, cells grown to confluence for seven days and supernatants collected

[g] MRL-*lpr* cell supernatant, cells grown to confluence for seven days and supernatants collected

[h] L-cell supernatant, L929 cell culture supernatants (24 hr)

[i] mouse rGM-CSF, 5 ng/ml

MC secrete M-CSF and not GM-CSF. M-CSF was originally purified from mouse fibroblast L cells [17]. M-CSF cDNA was cloned from and shown to be expressed by an L cell derivative, mouse fibroblast L929 cells [18]. Thus, these cells were used as a positive control. To determine specificity of CFU, MC supernatants or L929 supernatants were incubated with dilutions of anti-M-CSF or anti-GM-CSF. Colony formation was inhibited by dilutions of anti-M-CSF (>1:1000), but not by anti-GM-CSF (1:20). Similar results were obtained using a proliferation assay (Table 4). Proliferation of normal bone marrow induced by C3H MC or MRL-*lpr* MC supernatant was completely blocked by anti-M-CSF, but not anti-GM-CSF. To ensure specificity, anti-M-CSF (1:100) was neutralized by incubating with L929 SN from confluent L929 cells after six days of growth. Cells were then stained with anti-M-CSF or with the neutralized antibody at the same dilution of anti-M-CSF. A 1:1 mixture of L929 SN: anti-M-CSF (1:100) blocked detection in the C3H MC (68% to 9%) and MRL-*lpr* MC (28% to 14%).

MC express a 31 kDa form of M-CSF. Immunoprecipitated cell lysates from L929 cells (lane 2), C3H, MRL-*lpr* MC (lanes 3, 4) but not CH33 tumor cells (lane 1) expressed a prominent 31 kDa band (Fig. 3). In addition to the L929 cell lysate as a positive control, L929 supernatant was also electrophoresed and blotted (lane 7). Faint bands are visible at 31 kDa. Additionally, non-specific bands at 49 and 58 kDa are seen in all the lanes of the immunoprecipitated cell lysates demonstrating that protein was loaded in all these lanes and available for specific binding to the anti-M-CSF. In a separate experiment (not shown) using ^{125}I-goat anti-rabbit antibody and autoradiography of immunoprecipitated and non-immunoprecipitated cell extracts, a 16 kDa species was also evident in the extracts from L929 cells, C3H MC and MRL-*lpr* MC, but not from the CH33 B cell line.

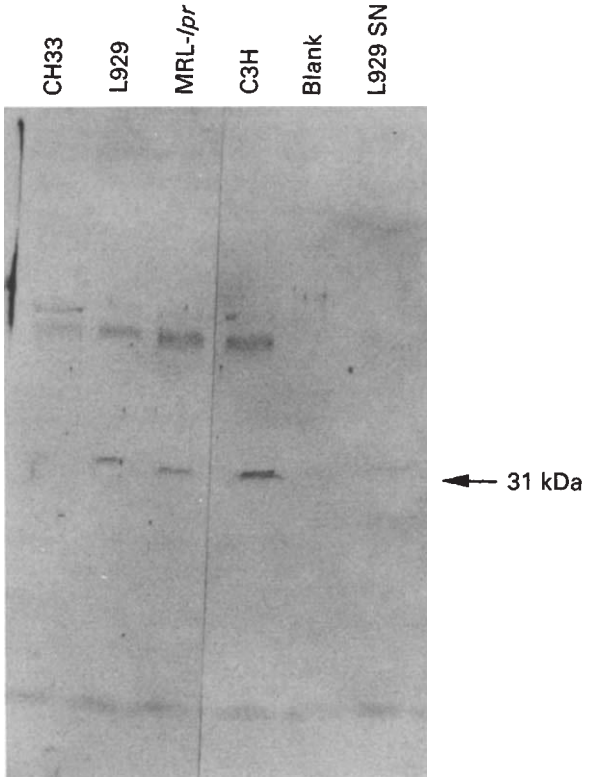

Fig. 3. *Mesangial cells express a 31 kDa form of M-CSF.* Cell lysates from C3H, MRL-*lpr*, MC, L929 and CH33 cells, and L929 supernatant were analyzed by Western Blot. Non-specific bands of approximately 49 and 58 kDa are evident in all cell lysate lanes (lanes 1–4), indicating similar levels of protein were loaded. Specific bands are present at 31 kDa in the MC and L929 cell lysates as well as the L929 SN. These are not present in the CH33, the negative cell type.

Discussion

Systemic lupus erythematosus is a devastating illness associated with a mesangioproliferative glomerulonephritis. MRL-*lpr* mice, a murine model of SLE, develop mesangioproliferative glomerulonephritis with macrophage infiltration into the glomerulus and an increased expression of TNFα and IL-1β transcripts [1, 2]. The MRL-*lpr* strain has extemely high levels of circulating M-CSF at one week, long before disease is present [5]. At older ages the source of the increased M-CSF is in part attibutable to the kidney. Because MC are known to make M-CSF [6, 7] and because MC proliferate in glomerulonephritis, we investigated whether any intrinsic differences existed between cultured MC from normal and MRL-*lpr* mice, specifically whether MRL-*lpr* MC could account for the increase in renal M-CSF. Unexpectedly, cultured MC from MRL-*lpr* mice secreted and expressed less M-CSF basally than MC from C3H mice. TNFα stimulation doubled M-CSF secretion by MRL-*lpr* MC and did not increase secretion of M-CSF by C3H MC. Total levels from the cultured MRL-*lpr* MC stimulated with TNFα, however, were only modestly higher than those from the C3H MC. Membrane M-CSF was not increased with TNFα stimulation in either of the cell lines. However, since there are more MC in MRL-*lpr* with nephritis than in normal mice, an increase in MC number may be responsible for the enhanced generation of M-CSF.

This study highlights the novel observation of surface M-CSF expression on non-transfected cells. Although others have demonstrated cell surface M-CSF on transfected COS cells [19], our studies are the first to report cell surface M-CSF on MC. In addition, these studies point out a difference between surface M-CSF on MC from normal mice and from mice with autoimmune disease. It is unlikely that this cell membrane M-CSF is anything other than surface bound M-CSF for several reasons. The positive control L929 cells, from which M-CSF was first purified, expressed elevated levels of M-CSF that were similar to the C3H MC and more than the MRL-*lpr* MC by flow cytometry. A negative cell type, the CH33, had virtually no detectable M-CSF. It is also unlikely that we detected M-CSF that had been secreted and then bound to the M-CSF receptor. The 16 and 31 kDa forms of M-CSF we detected on Western blot are consistent with previous reports in other cell types [17, 20], and inconsistent with simultaneous detection of M-CSF bound to the M-CSF receptor. Further evidence against this possibility is as follows: (1) L929 cells lack c-*fms*, the M-CSF receptor [21]; (2) the same size moiety (31 kDa) on Western blots of cell lysates were seen in the L929 cells and the MC; (3) the fluorescence patterns by flow cytometry were seen in the L929 cells and MC. Although MC have been reported to express c-*fms* and to bind M-CSF [7], since the number of copies of the receptor expressed is extremely small, it is unlikely c-*fms* expression could account for the density of M-CSF observed as reflected by the intensity of staining and mean log fluorescence. Finally, we were unable to detect c-*fms* in our cells by Northern blot analysis (not shown). However, it is possible that the RNA probed had below detectable levels since we did not prepare poly (dt) purified RNA as previously reported [7].

Although expression of M-CSF by MC and in the circulation of autoimmune mice could be an epiphenomena, we favor the suggestion that M-CSF is central to the pathogenesis of lupus nephritis in MRL-*lpr* mice. M-CSF is a macrophage chemoattractant [1] as well as a growth and differentiation factor [reviewed in 2]. MRL-*lpr* MC reside in a milieu in which there is increased TNFα [3, 4]. TNFα is known to stimulate M-CSF in other cell types [22, 23], and we have shown that TNFα increased secretion of M-CSF in MRL-*lpr* MC. Increased renal M-CSF and possibly circulating M-CSF may be secondary to the increased renal TNFα. Therefore, cytokine stimulation may enhance the production of M-CSF by MC and in turn recruit macrophages to the glomerulus. Alternatively, macrophages may be attracted to the mesangium by other stimuli and then stimulate MC to generate M-CSF. This mechanism may not be operative in all forms of renal injury. Another autoimmune strain, the NZB/W F1 female hybrid does not have elevated levels of circulating M-CSF at any age [5]. Of note, the MC from these NZB/W mice have a secreted and surface expression of M-CSF similar to the normal C3H strain (unpublished data). This is not surprising since the expression of lupus is very distinctive in these models and there are few established common denominators.

A less obvious and more intriguing feature of M-CSF in MRL-*lpr* mice lies in the fact that M-CSF and stem cell factor (SCF) share genetic and structural homology [24], suggesting a

more fundamental role for M-CSF in cell growth and regulation. As further support of this expanded role, M-CSF has been found to act as a "start factor" to stimulate cyclins and move cells from the G_1 to S phase of the cell cycle [25]. Thus, M-CSF may play a role locally in the proliferation of macrophages and other cell types. In fact, recent experiments in our own laboratory indicate that M-CSF is responsible for stimulating glomerular macrophages to divide as well as maintaining their viability. Further studies exploring this action of M-CSF within the kidney are in progress.

Finally, we appreciate that these studies, which are restricted to the examination of cultured mesangial cells, may not reflect the expression of MC M-CSF in vivo. In fact, since there is enhanced cytokine levels, immunoglobulins, and a myriad of other molecules in the renal cortex and circulation of MRL-*lpr* mice as compared to normal mice, the environment will certainly influence M-CSF expression. We are currently extending our studies and examining MC in renal tissues of MRL-*lpr* mice for both mRNA transcripts and the production of M-CSF.

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